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Effects of Fly Ash and Its Constituents on Sensory Irritation in Mice

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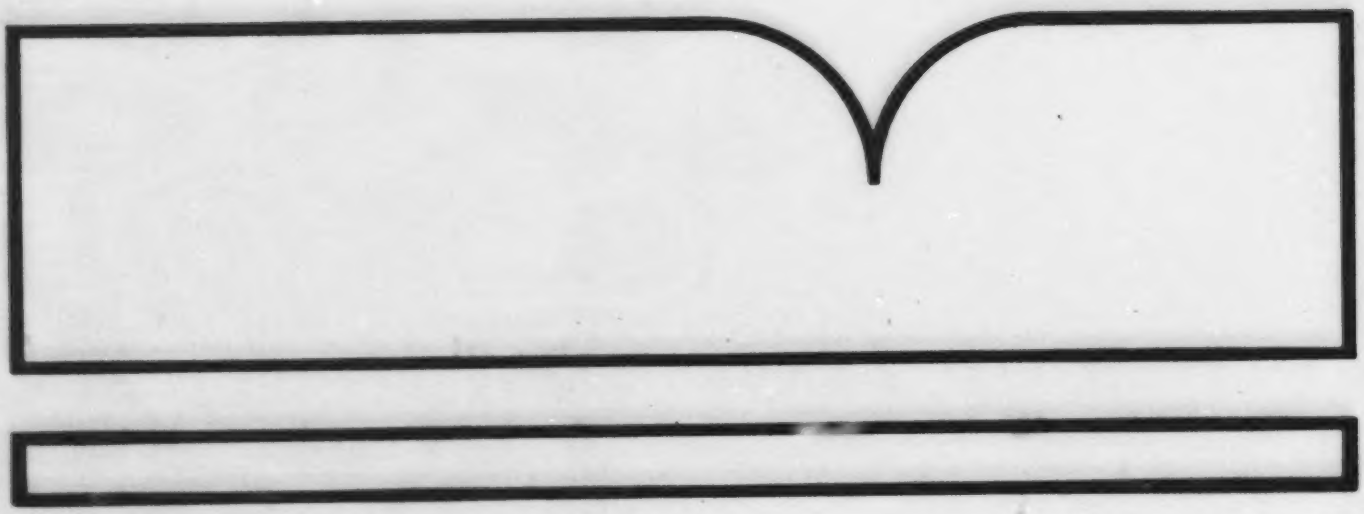

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16. ABSTRACT

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Effects of Fly Ash and its Constituents on Sensory Irritation in Mice

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ABSTRACT

Effects of Fly Ash and its Constituents on Sensory Irritation in Mice. Hatch, G.E., Boykin, E., Miller, F.J. and Graham, J.A. (1982). *Fundam. Appl. Toxicol.* 2:77-81. Sensory irritation caused by fly ash from oil-fired (OF), fluidized bed coal (FB) combustion and conventional coal (CC) combustion power plants, and from Mt. St. Helens volcano (VA) was studied in mice. The irritating sensation due to contact with fly ash or its constituents was quantitated by observing a characteristic flexing of the dorsal musculature of the mouse following intraperitoneal injection of the ash suspension. Construction of dose-effect curves was based on the percentage of animals showing a positive response at each ash concentration. The order of irritant potency of fly ash samples was as follows: OF and OF leachate $\gg$ FB $>$ CC $>$ VA. Saline leachates of ash samples other than OF fly ash showed no irritating effects. OF fly ash was about 160× more irritating than VA and 5× more irritating than the reference detergent compound, sodium dodecyl sulfate. Study of chemicals known to be present in fly ash or chemicals similar to those present indicated that acidic or basic compounds, heavy metal ions and metal oxides, and insoluble particles could all contribute to the irritancy of an ash. Soluble heavy metals appeared to be mainly responsible for the irritant effects of OF ash, while insoluble particles including metal oxides could have accounted for the irritancy of the other fly ash samples studied. Comparison of data from the peritoneal irritation test used here with previously published data from the upper respiratory tract irritation test of Alarie confirmed the previously noted correlation between these two tests for chemicals in solution.

INTRODUCTION

The goal of the present study was to determine the relative irritancy of small samples of coal and oil combustion fly ash. Methods used to date for measuring the irritancy of substances have fallen into two categories: 1) tests which attempt to quantitate tissue damage and the accompanying inflammatory response (primary tissue irritation), and 2) tests which attempt to quantitate the pain or sensory response associated with exposure to the irritating chemicals (sensory irritation). The Draize eye irritation test, which falls into the former category, has been used for many years in standard protocols for determining chemical irritation (Draize *et al.*, 1944). In the category of sensory irritation tests, Alarie (1966) developed and applied a technique in which stimulation of the upper respiratory tract by an inhaled irritant produces a dose-related inhibition of respiratory rate. Excellent agreement was observed between the relative irritant potency in mice using this test and subjective human data on a large number of chemicals (Alarie, 1981). Another measurable sensory reflex to irritant chemicals was first described by Vander Wende and Margolin (1956) as "contraction of the abdominal musculature accompanied by writhing of the body, and backward extension of the hind limbs" following intraperitoneal injection of the irritant. The peritoneal irritancy test has since been used to evaluate surfactant and analgesic effects (Pearl *et al.*, 1968). Shanahan and Ward (1975) showed that the peritoneal irritancy test in mice yielded data correlative with human eye sting studies of 11 shampoos. Because all of these shampoos were innocuous in the Draize test it appeared that the sensory irritation test was more sensitive than the test which measured tissue damage directly. Later, Cuichta and Dodd (1978) were able to demonstrate that the effects of 7 surfactants on mouse peritoneal irritation were correlated with data obtained from the upper respiratory tract irritation test of Alarie and with the Draize test. However, the degree of correlation with the Draize test was limited by the lack of dose-effect behavior of the Draize test data. The latter test appeared to require higher concentrations of test materials to show a positive response than the peritoneal irritation test.

Apparently, none of the irritation tests have yet been applied to particulate materials. Also, no evaluation appears to have been made of the mouse peritoneal irritancy test for nonsurfactant chemicals. Reported here is an effort to apply the peritoneal irritancy test to fly ash samples and to materials which could be present in fly ash.

METHODS

Animals

Pathogen-free female mice (CD-1, COBS) weighing 18-23 g (approx. 4-5 weeks old), obtained from Charles River Breeding Labs, Wilmington, MA, were used in all experiments.

Chemicals and fly ash samples

Amyl acetate, ethyl acetate, acetaldehyde, paraformaldehyde, acrolein, and toluene diisocyanate were purchased from Aldrich Chemical Co., Milwaukee, WI; ferric oxide and powdered carbon from Pressure Chemical Co., Pittsburgh, PA; minusil silica (< 5 μm diameter by specification) from Pennsylvania Glass Sand Corp., Pittsburgh, PA; talc, kaolin, and ferric sulfate from Mallinkrodt Inc., Paris, KY. All other

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TABLE 1
Effect of Fly Ash Samples on Mouse Writhing Response

	WD50 (% by wt.)	95% Confidence Limits
Residual oil fly ash	0.010	0.007 - 0.0135
Fluidized bed coal fly ash	0.187	0.125 - 0.255
Conventional combustion		
Coal fly ash #1	0.357	0.290 - 0.454
Coal fly ash #2	0.682	0.50 - 1.07
Fine volcanic ash	1.67	0.610 - 2.62
Sodium dodecyl sulfate (positive control)	0.065	0.048 - 0.087

chemicals used were obtained from Fisher Chemical, Fairlawn, NJ. Chemicals were suspended or dissolved in 0.9% saline for injection (Abbott Laboratories, North Chicago, IL) at a 10% wt/vol concentration from which further dilutions were made. Particle sizes, measured by scanning electron microscopy on the bentonite and fly ash samples, showed 90% of the particles within the range of 0.2 - 6.0 μm diameter. The other particles used appeared to have similar size characteristics when examined by light microscope. Leachate (particle supernates) were prepared by centrifugation (20 000× G) following a 24 hr incubation at 25 °C of the fly ash suspensions. Leachate concentration was expressed in terms of percent wt/vol of the fly ash suspension from which it was derived. Toluene diisocyanate was dissolved in laboratory grade corn oil instead of saline.

Residual oil fly ash was collected by Southern Research Institute, Birmingham, AL, using a teflon-coated fiberglass filter downstream from the cyclone of a power plant in Florida which was burning a low sulfur #6 residual oil. The collection temperature was 250-300 °C. *Fluidized bed combustion fly ash* was collected by the Energy Assessment and Control Division, IERL, EPA, from an experimental boiler located at Research Triangle Park, NC. This boiler was burning Pittsburgh seam coal at 825 °C using Grove limestone as sorbent (Ca/S ratio = 3:1). The collection temperature was 218 °C. *Conventional coal combustion fly ash #1* was obtained from a power plant in Gorgas, Alabama using a glass fiber filter located downstream from the in-plant electrostatic precipitator and cyclone. The particle size was < 2.5 μm. The collection temperature was 135 °C. *Conventional coal combustion fly ash #2* was collected by Exxon Research and Engineering Company, Linden, NJ. This ash was taken from the hopper of the last plate on an electrostatic precipitator in a 200 megawatt plant burning Eastern Kentucky Coal. The collection temperature was approximately 140 °C. The hopper ash was further fractionated to obtain a sample with < 3.3 μm particle size. The elemental and organic composition of this ash has been determined previously (Griest and Guerin, 1979). *Mt. St. Helens Volcanic Ash* was collected for EPA by Dr. Andrew McFarland, Texas A&M Univ. from an open field near Ritzville, WA (340 km northeast of the volcano) on June 8, 1980. It was fractionated to obtain the sample used here which had a mean particle size of < 2.5 μm.

Assessment of the peritoneal irritancy response

Each mouse was restrained in a plexiglass holder, held by the tail and injected intraperitoneally with 0.2 mL of solution in the mid-ventral surface of the abdomen (Shanahan and Ward, 1975). Needles (25G, 7/8 in) were changed every 2-3 mice so that a sharp needle was always used. Mice were rejected from the experiment if the injection appeared to be subcutaneous or drew blood. After injection, the mouse was placed in a box with opaque walls and observed for up to 3 min. Mice showing an irritant response were euthanized immediately and all others after 3 min. The irritant response was defined as contraction of abdominal and back musculature so that indentations were visible on the dorsum of the mouse. Other responses to pain were sometimes also present, however, we found that observing only this sign caused dose-effect curves to fall more steeply to zero as concentration of irritant was decreased. Stringent observation of this sign also seemed to lower the chances for misinterpretation of the response. Room temperature appeared to be important. Lower temperatures caused piloerection which made the indentations of the back less visible. All experiments in this study were performed at room temperatures between 75 and 80 °F. The amount of variation that might occur between different investigators was determined using a purified irritant chemical, sodium dodecyl sulfate. Four different experimenters at widely spaced intervals (weeks-months) independently obtained the following median effective writhing dose (WD50) values for this compound: 0.065%, 0.047%, 0.050%, and 0.056%.

Statistics and experimental design

Preliminary range finding was accomplished by injecting 10-fold dilutions starting at 10% wt/vol and going down using 5 mice at each concentration. The approximate range in which responses became negative was determined and further injections carried out within this range using at least 10 mice for each concentration tested. When at least 3 points showed intermediate percent writhing (10-90%) the WD50 and confidence limits were determined by probit analysis using the POLO program of Russell *et al.* (1977). Significant differences in WD50 values were determined from the lack of overlap of 95% confidence limits.

RESULTS

Effects of fly ash samples on the mouse writhing response

Five samples of fly ash obtained from different types of coal and oil combustion were compared with each other and with volcanic ash from Mt. St. Helens in their ability to induce sensory irritation when injected intraperitoneally in mice (Table 1). The most irritating ash was that obtained from the residual oil power plant. This ash was almost 20 times as potent as the next most irritating ash, a sample of fluidized bed combustion coal fly ash, and about 160 times more irritating than Mt. St. Helens volcanic ash. Sample #1 of the fly ash from conventional coal combustion was significantly more irritating than Mt. St. Helens volcanic ash, while sample #2 was not. All other ash samples except the later two differed significantly ($p < .05$) from each other in their irritant potency.

Effects of leachates from fly ash and contribution of pH

Suspension of fly ash in unbuffered physiological saline resulted in an acidic solution in the case of residual oil fly ash, and a basic solution in the case of fluidized bed combustion fly ash (Table 2). Conventional coal combustion fly ash changed the pH of saline only slightly from 5.2 to approximately 4. Titration of the suspensions indicated that the former two ashes contained about 0.7 milliequivalents per gram of ash of base (fluidized bed) or acid (residual oil fly ash).

The effect of pH on irritancy of a saline solution was determined by adjusting the pH with HCl or NaOH. Saline having a pH between 2.8 and 11.2 had no detectable irritating effect. Sharp increases in irritancy were seen as further extremes of pH were reached.

Experiments with fly ash leachates were performed in order to determine whether soluble or insoluble components of the fly ash were responsible for the writhing response to the ash. The residual oil fly ash leachate was equally as irritating as the whole fly ash suspension (WD50 of leachate was 0.011% with 90% confidence limits of 0.005 to 0.015). None of the other fly ash leachates prepared from 1% suspensions of ash showed any irritating activity. Table 2 shows the expected irritant response based on the pH of the fly ash leachates. Since the leachate from residual oil fly ash had a much greater irritating effect than could be accounted for by its low pH, it appeared that pH was a minor determinant of the irritancy of this ash. The pH of the leachates from the other ash samples did not appear to be extreme enough to produce irritation.

Effects of possible fly ash constituents on the mouse peritoneal irritancy test

The possible contributions of insoluble silicate or carbonaceous material, as well as oxides and soluble salts of heavy metals were investigated by examining the irritancy of some well-characterized materials. Results shown in Table 3 indicate that bentonite was the most potent irritant of the insoluble mineral particles studied, having a WD50 of approximately 0.1% which was roughly in the same category as fluidized bed coal fly ash. Talc appeared to be slightly less irritating than bentonite, and carbon, kaolin and silica powders were even less irritating. Bentonite was approximately 40 times more irritating than the least potent particle, silica.

Since metallic compounds are known to be present in fly ash, we examined the irritating effects of 10 soluble metal salts, and followed this with an investigation of 7 of the metal oxides. Results depicted in Table 3 show that many of the soluble heavy metals studied were considerably irritating. Cadmium followed by nickel chlorides were the most potent irritants studied, with WD50's in the .001-.01% range. Ammonium and sodium sulfates were the least irritating of the salts studied. Other compounds which exhibited intermediate effects were Na_3VO_4, $CuSO_4$, $VOSO_4$, $Fe_2(SO_4)_3$, $Pb(NO_3)_2$, and $ZnSO_4$.

Oxides of the heavy metals usually had considerably less irritating effect than the parent metal ion. NiO, CuO, and Fe_2O_3 exhibited no measurable irritating effect at levels up to 3% wt/vol of ash (Table 3). Cadmium oxide was the most irritating of the oxides, having a WD50 of about .006%. Calcium and zinc oxides also showed considerable irritating activity with WD50 values of 0.05% and 0.15% respectively. Leachates of these oxides were much less irritating than the original suspension and their effects could have been totally due to their high pH (Table 2). Although sodium vanadate and vanadyl sulfate solutions exhibited high and low pH values, respectively, it did not appear that the pH of these solutions was extreme enough to significantly contribute to their irritancy.

Comparison of the mouse peritoneal irritancy test with the upper respiratory tract irritation test

Ten compounds previously studied by Alarie were injected into mice at different dosage levels to determine the molar WD50. WD50 values are shown in Figure 1 plotted against the air concentrations of the same chemicals which were 50% effective in reducing the respiratory rate (RD50) in exposed mice (Alarie, 1981). Linear regression analysis yielded a correlation coefficient of 0.949 ($p < 0.001$). The upper respiratory tract irritation test yielded a wide spread of RD50 values which spanned 6 orders of magnitude of air concentrations, while WD50 values from mouse writhing data are contained within 4 orders of magnitude of injected concentrations. No particles were included in the correlation because of the lack of data available on particles in the respiratory irritancy test.

DISCUSSION

Results seen using the mouse writhing test suggest that fly ash can be very irritating. The fly ash sample obtained from a residual oil combustion boiler, for example, was almost 6 times more active on a weight percent basis than a representative detergent powder, sodium dodecyl sulfate (Table 1). The

TABLE 2
Contribution of Hydrogen Ion Content to the Writhing Response of Ash and Ash Constituent Leachates

Substance	Leachate Conc. (% by wt. of original suspension)	pH	Percent Response: Expected Based on pH	Percent Response: Observed
Ash Samples:				
Residual oil fly ash	10.0	2.4	10	100
Residual oil fly ash	1.0	2.7	2	100
Coal fly ash #2	1.0	3.9	0	0
Coal fly ash #1	1.0	4.2	0	0
Volcanic ash	1.0	6.3	0	0
Fluidized bed fly ash	10.0	11.3	5	0
Possible Ash Constituents:				
$VOSO_4$	0.1	2.4	10	80
ZnO	10.0	7.7	0	0
CdO	1.0	10.8	0	0
Na_3VO_4	0.1	11.3	5	100
CaO	10.0	12.5	80	100
CaO	3.0	12.0	45	40

TABLE 3
Effects of Metals, Metal Oxides, and Insoluble Particles on the Mouse Peritoneal Irritation Test

Substance	No. Mice	WD50 (% wt/vol)	95% Conf. Limit
$CdCl_2$	50	0.0015	
$NiCl_2$	50	0.0019	0.0016 - 0.0070
CdO	70	0.0045	0.0026 - 0.0070
Na_3VO_4	30	0.020	
$CuSO_4$	40	0.044	0.027 - 0.069
$VOSO_4$	30	0.062	0.038 - 0.097
$Fe_2(SO_4)_3$	30	0.061	
CaO	40	0.071	0.043 - 0.167
$Pb(NO_3)_2$	40	0.095	
Bentonite	60	0.112	0.096 - 0.127
$ZnSO_4$	40	0.173	0.108 - 0.279
Talc	50	0.318	0.118 - 0.511
ZnO	90	0.790	0.358 - 1.73
$(NH_4)_2SO_4$	40	1.13	0.722 - 1.70
Carbon	30	1.50	
Kaolin	30	1.55	0.994 - 2.42
Mn_3O_4	40	1.76	1.58 - 5.26
Na_2SO_4	40	3.94	1.87 - 5.42[A]
Silica	30	4.36	2.71 - 7.17
NiO	30	>4.0	
CuO	30	>4.0	
Fe_2O_3	30	>4.0	

[A] 90% confidence limit

residual oil fly ash sample was unusual in that water soluble components of the ash were responsible for all of its irritating activity. Two samples of conventional coal combustion fly ash and one sample of fluidized bed combustion coal fly ash were less irritating than the residual oil fly ash, and the leachates of these ashes did not possess any irritating activity. The fluidized bed combustion fly ash appeared to be more irritating than ash from conventional coal combustion.

Unbuffered saline suspensions of fly ash were used since little buffering of the ash would be expected if it were to impinge on mucous membranes. The residual oil fly ash had a high acid content and the fluidized bed fly ash a high base content, while the other samples were close to neutrality. However, results of irritation tests versus pH of solutions discounted the contribution of hydrogen or hydroxide ions in fly-ash-induced irritation. Significant irritating effects of these ions only occurred at extreme values of pH (< 2.8 or > 11.2) which were only reached at higher fly ash concentrations than those which showed irritating properties (Table 2).

Some suspended particles were shown to be irritating in the mouse peritoneal injection system, and large differences in irritancy were seen between particles even when the size of the particles was similar. All of the irritation caused by the volcanic ash and the fluidized bed and conventional coal combustion fly ashes could have been accounted for by the insoluble particulate material. This conclusion was based on the lack of writhing activity seen in mice injected with leachates from these ash samples, and by the fact that other water-insoluble particles such as talc, bentonite, and metal oxides were able to elicit a writhing response at concentrations comparable to those of the fly ash samples. It appeared from Table 3 that among the most potent irritant particles might be the oxides of metals such as cadmium and calcium. Concerns have been expressed that fluidized bed combustion might result in a more irritating fly ash because of calcium oxide released from the limestone sorbent during combustion. This idea is supported by the high irritant effect seen here due to fluidized bed ash in comparison with conventional coal fly ash, and by the fact that the former ash produced suspensions of high pH with highly irritating particulate material such as is seen with pure calcium oxide.

Experiments with soluble metal salts indicated that even small amounts of some of these materials could greatly influence the irritancy of a fly ash sample. Results from atomic absorption analysis of the fly ash leachates suggested that at least one soluble metal could be an important contributor to the irritancy of the residual oil ash. The leachate of a 10% wt/vol suspension of this ash contained 333 ppm of Ni, while none of the other ash samples contained greater than 2 ppm of this metal. The irritating effects of soluble components of the fly ash appeared to be extrapolable to respiratory irritancy due to the correlation observed here between the peritoneal irritation test and the upper respiratory irritancy test of Alarie. This correlation was demonstrated previously for surfactants (Ciuchta and Dodd, 1978). Such a correlation may indicate a similarity in the chemical responsiveness of nociceptors in the peritoneal cavity and in the nasopharynx.

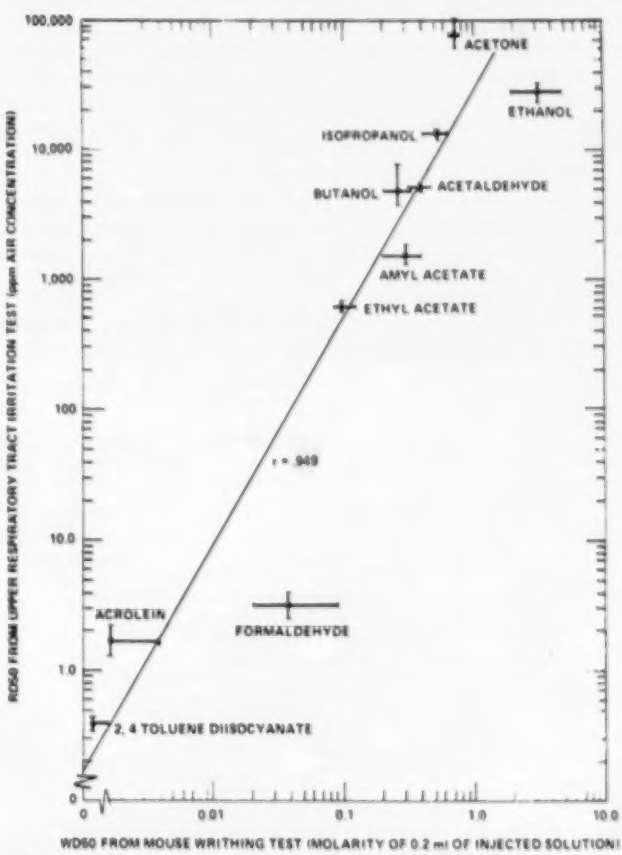

FIG. 1. The irritancy of 10 chemicals assessed by two tests of sensory irritation: upper respiratory tract irritation test (ordinate) and mouse writhing test (abscissa). Ninety-five percent confidence limits are shown for each chemical. Values for the abscissa were taken from Alarie (1981). The equation for the line drawn by linear regression analysis is $\log y = 4.39 + 1.70 \log x$.

The mouse peritoneal sensory irritation test appears to be a sensitive and rapid method which can be used to gather information on environmentally important materials. The sensitivity of the test is illustrated by the WD50 of cadmium chloride which translates to a dose of 0.15 mg/kg. The peritoneal test is simpler to perform and requires less sample than the upper respiratory tract irritancy test because the latter test requires aerosolization and monitoring of the exposure compounds. As seen here, the peritoneal test appears to be useful for screening chemicals to determine priorities for higher level studies, and also for determining the active components of complex mixtures.

A disadvantage of presently available tests of chemical irritation is that they all inflict some degree of pain in the experimental animal used. Primary irritation tests especially can cause long-term animal suffering (Rowan, 1981). The peritoneal test seems to induce a minimum of stress because it requires only a few seconds of observation after which animals are euthanized. The number of animals used and the severity of the responses seen could be further reduced by observation according to an up and down format (Dixon and Massey, 1957). Several questions remain to be answered concerning the extent to which sensory irritation tests are predictive of or could be substituted for tests of primary tissue irritation. Also, it is possible that *in vitro* tests could be developed which will yield data predictive of *in vivo* chemical irritation.

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